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Partial characterization of the N-linked oligosaccharide structures on P-selectin glycoprotein ligand-1 (PSGL-1)

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ABSTRACT

PSGL-1, a specific ligand for P-, E- and L-selectin, was isolated from in vivo [^{3}H]-glucosamine labeled HL-60 cells by a combination of wheat germ agglutinin-agarose and P- or E-selectin-agarose chromatography. N-linked oligosaccharides were released from the purified, denatured ligand molecule by peptide: N-glycosidase F treatment and, following separation by Sephacryl S-200 chromatography, partially characterized using lectin, ion-exchange and size-exclusion chromatography in combination with glycosidase digestions. The data obtained suggest that the N-glycans on PSGL-1 are predominantly core-fucosylated, multiantennary complex type structures with extended, poly-N-acetyllactosamine containing outer chains. A portion of the outer chains appears to be substituted with fucose indicating that the N-glycans, in addition to the O-glycans on PSGL-1, may be involved in selectin binding.

Key words: *Selectin ligands, PSGL-1, N-glycans*

INTRODUCTION

P-, E- and L-selectin are C-type lectins involved in the binding of circulating leukocytes to various target cells[1], [2]. The interaction(s) between the selectins and the target cells is mediated by oligosaccharide structures conjugated to specific ligand molecules expressed on the target cell surfaces. These ligand molecules may be recognized by one, two or all three of the selectins[1-3]. Although available data suggest that the interaction(s) between the selectins and their ligands in most cases is mediated by O-linked oligosaccharide structures, specific binding of E-selectin to N-linked oligosaccharides has been reported[4-6].

PSGL-1 is a ligand capable of binding P-, E- and L-selectin[7-13]. The molecule is expressed on several cell types, including human leukocytes and the human myeloid cell line HL-60[7], [10], [14]. Moreover, successful cloning from an HL-60 cDNA library has revealed that PSGL-1 is a type I membrane glycoprotein with a domain structure similar to that of many cell surface mucins[9], [15]. The amino acid sequence contains a "mucin domain" composed of 15 serine/threonine rich decameric repeat sequences that are believed to be extensively substituted with O-linked oligosaccharides[9], [10], [16]. PSGL-1 is expressed only in quite limited quantities on the surface of ligand-carrying cells, hence purification in amounts sufficient for characterization of oligosaccharide structures at individual sites it not practical using current technology[17]. Nevertheless two characterizations of the

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Abbreviations: PSGL-1, P-selectin glycoprotein ligand-1; Gal, galactose; GlcNac, N-acetylglucosamine; LacNAc, N-acetyllactosamine; Man, mannose; NP-40, nonidet P-40; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; AAL, Aleuria aurantia lectin.

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total spectrum of O-linked oligosaccharides on HL-60 PSGL-1 have been reported[18,19] . And, based on current knowledge about P-selectin specificity, it appears likely that this lectin interacts with (one of) the minor, fucosylated O-glycans identified by these investigators.

In addition to the O-linked structures discussed above, the PSGL-1 sequence also contains three sites for N-glycosidic glycosylation and results from previous studies suggest that the molecule may contain significant amounts of N-linked oligosaccharides[9]. The nature of these structures, however, has not been determined.

This report presents a partial characterization of the N-linked oligosaccharides conjugated to HL-60 PSGL-1. The general types of structures are determined and the occurrence and distribution of fucose substitutions, is investigated.

MATERIALS AND METHODS

Materials

D-[6-^{3}H]Glucosamine hydrochloride (32 Ci/mmol) and Concanavalin A-Sepharose were purchased from Amersham Pharmacia Biotech. BioGel P-6 was from BioRad. Peptide: N-glycosidase F was from Genzyme. RPMI Medium 1640, heat-inactivated fetal bovine serum, trypsin, penicillin and streptomycin were from GibcoBRL. Arthrobacter ureafaciens neuraminidase, almond meal a-fucosidase and chicken liver a-fucosidase were from Oxford GlycoSciences. Bacteriodes fragilis endo-β-galactosidase and Pronase were from Roche. Aleuria aurantia (AAL) lectin was from Seikagaku. Vibrio cholerae neuraminidase, jack bean β-galactosidase and jack bean b-N-acetylglucosaminidase were from Sigma. All other supplies were from standard sources.

Metabolic labeling, isolation of the ligand molecule and preparation of radiolabeled oligosaccharides and glycopeptides

Labeling of HL-60 cells with [^{3}H]-glucosamine (50 μCi/ml) was carried out in normal RPMI medium for 48 h. The radiolabeled ligand molecule was isolated as outlined previously[10], [20].

The purity of the ligand molecule was routinely determined on SDS-PAGE; the purified preparations contained only one radioactive protein band[19].

N-linked oligosaccharides were isolated by peptide: N-glycosidase F digestion of the purified, SDS-denatured ligand molecule followed by Sephacryl S-200 chromatography, essentially as described by Roux et al.[21].

Glycopeptides were prepared from the intact purified radiolabeled ligand molecule by digestion with Pronase (0.5 mg/ml) in 0.1 M Tris-HCl, pH 8.0, 40 m M $CaCl_2$ at 37 °C for 24 h.

Enzyme treatments

Digestion with A. ureafaciens neuraminidase (2 U/ml) was done in 100 mM sodium acetate, pH 5.0 for 18 h; V. cholerae neuraminidase (250 mU/ml) was in 100 mM sodium acetate, pH 4.6, 150 mM NaCl, 10 mM $CaCl_2$ for 72 h; jack bean b-galactosidase (400 mU/ml) was in 50 m M citrate-phosphate, pH 4.6 for 48 h; jack bean b-N-acetylglucosaminidase (5 U/ml) was in 50 m M sodium citrate, pH 5.6 for 18 h; almond meal a-fucosidase (0. 2 mU/ml) was in 100 m M sodium citrate, pH 6.0 for 18 h; chicken liver a-fucosidase (2 U/ml) was in 100 mM sodium citrate-phosphate, pH 6.0 containing 250 mg/ml BSA and 100 mM NaCl for 18 h. Bacteroides fragilis endo- β-galactosidase (250 mU/ml) was in 50 m M sodium acetate, pH 5.6 for 18 h. Peptide: N-glycosidase F (10 U/ml) was in 100 mM Tris-HCl, pH 8.6, 0. 1% SDS, 1.5% NP-40, 10 mM 1, 10 phenanthroline for 24 h. All enzyme digestions were carried out at 37°C under a toluene atmosphere.

Lectin chromatography

Fractionation of in vivo [^{3}H]-glucosamine labeled PSGL-1 glycopeptides on Concanavalin A-Sepharose (0.8 ml bed volume) was done essentially as described by Merkle and Cummings[22]. Bound glycopeptides were sequentially eluted with 10 mM a-methylglucoside followed by 100 m M a-methylmannoside at 55 °C; fraction volume was 2 ml. Fractionation on Aleuria aurantia lectin-Sepharose (0.5 $\times$ 10 cm) was done essentially as described [23]. Column buffer was 10 mM sodium phosphate, pH 7.4, 150 m M NaCl. Samples were allowed to interact with the column for 30 min prior to elution in the column buffer. Bound material was eluted with 1 mM fucose; fraction volume was 1 ml; flow rate was 15 ml/h.

Ion-exchange chromatography

Ion-exchange chromatography on QAE-Sephadex was carried out essentially as described by Varki and Kornfeld[24]. Charged oligosaccharides were eluted step-wise with increasing concentrations of NaCl in 2 m M Tris base; 1.5 ml fractions were collected; the column bed volume was 1 ml. Sialic acid was removed by treatment with A. ureafaciens neuraminidase.

Size exclusion chromatography

Size exclusion chromatography of SDS-solubilized PSGL-1 on Sephacryl S-200 was done on a 0.7 $\times$ 50 cm column equilibrated in 10 m M Tris-HCl, pH 6.5 containing 0.2% SDS[18]; 50 approximately 700 μl (26 drops) fractions were collected.

Size exclusion chromatography and sequential exoglycosidase digestion of desialylated oligosaccharides was carried out on an Oxford GlycoSciences GlycoSequencer using a Biogel P-4 type column (Glycan Sizing Column, Oxford GlycoSciences) and the High-Resolution Program (30 μl/min for 366 min followed by a linear increase to 200μl/min over 234

min); 2 drop fractions (approximately 90 μl) were collected.

RESULTS

Glycopeptides, prepared by Pronase digestion of the intact [^{3}H]-glucosamine labeled ligand molecule, were separated on Concanavalin A-Sepharose (Fig 1). This resulted in essentially all of the applied radioactivity eluting in the breakthrough fractions from the column and only insignificant amounts eluting by the hapten sugars a-methylmannoside and α-methylglucoside, suggesting that PSGL-1 does not contain significant amounts of high-mannose, hybrid or biantennary complex type oligosaccharides[25].

Treatment of SDS-denatured in vivo [3H]-glucosamine labeled PSGL-1 with peptide: N-glycosidase F, followed by separation of the reaction products by Sephacryl S-200 chromatography, resulted in a significant portion, between 20% and 37% in different preparations, of the radioactivity eluting at an apparent lower molecular size than the intact glycoprotein (Fig 2, panel A). Strong acid hydrolysis of this peptide: N-glycosidase F released radioactivity, followed by separation of the resulting free amino sugars on paper chromatography, resulted in the recovery of only N-acetylglucosamine (Fig 2B, inset), suggesting that the peptide: N-glycosidase F released material represents N-linked oligosaccharides only, and does not contain any O-linked oligosaccharides (Compare compositional analysis of the total oligosaccharides on PSGL-1[19]. These observations are consistent with approximately one fourth of the radioactivity incorporated into [^{3}H]-glucosamine labeled PSGL-1 being associated with N-linked structures. The radioactivity eluting in the included peak (centered around fraction 31) in the separation of the undigested material (Fig 2, panel A) was identified as free glucosamine by paper chromatography (data not shown). Retreatment of the radioactivity in the earlier eluting peak from the separation of the peptide: N-glycosidase F treated material (Fig 2, panel B), with peptide: N-glycosidase F, did not result in any further release of radioactivity, suggesting that all susceptible structures were cleaved from the molecule by the initial treatment.

In order to obtain structural information about the N-glycans on PSGL-1, a portion of the peptide: N-glycosidase F released radioactivity was digested

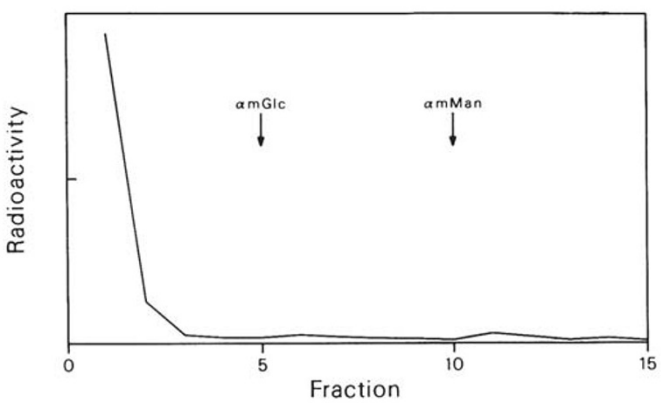

Fig 1. Concanavalin A-Sepharose chromatography of glycopeptides isolated from labeled PSGL-1. Purified, in vivo [^{3}H]-glucosamine labeled PSGL-1 was digested with Pronase and the resulting glycopeptides were separated on Concanavalin A-Sepharose as outlined in Materials and Methods. Bound radioactivity was eluted sequentially with a-methylglucoside (amGlc) and a-methylmannoside (amMan)

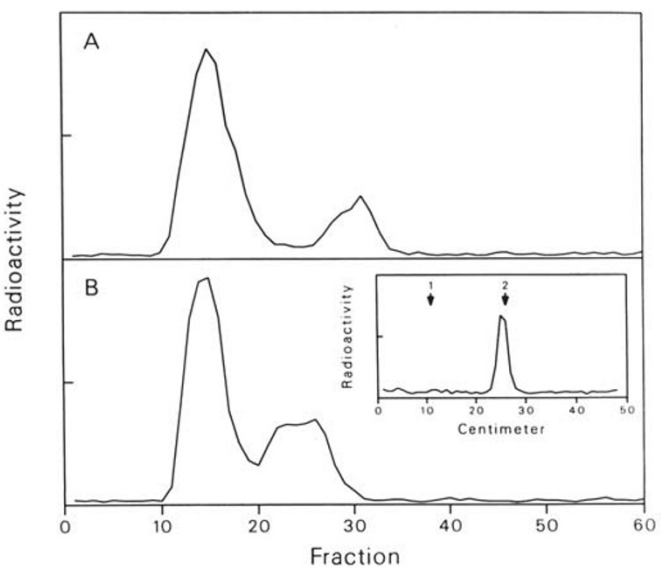

Fig 2. Size-exclusion chromatography of PSGL-1; removal of N-linked oligosaccharides. Purified, in vivo [^{3}H]-glucosamine labeled PSGL-1 was denatured in SDS and separated on a Sephacryl S-200 column (0.7 $\times$ 50 cm) equilibrated in 10 m M Tris-HCl, pH 6.5, 0.2% SDS. Panel A, untreated, denatured PSGL-1. Panel B, re-chromatography of fractions number 12 through 18 from the separation shown in panel A, following extensive treatment with peptide: N-glycosidase F. Panel B, inset: paper chromatography of products generated by strong acid hydrolysis of an aliquot of the radioactivity released by peptide:N-glycosidase digestion of PSGL-1. The migration positions of standards are indicated: 1, N-acetylgalactosamine; 2, N-acetylglucosamine.

with Arthrobacter ureafaciens neuraminidase and separated on BioGel P-6. This resulted in the profile shown in Fig 3. Approximately 31% of the radioactivity eluted as a well-defined peak close to Vi. This radioactivity was identified as sialic acid by paper chromatography (Data not shown). Moreover, separation of the total peptide: N-glycosidase F released oligosaccharides on QAE-Sephadex revealed that PSGL-1 contains a mixture of non-sialylated and sialylated structures: Approximately 30% of the applied radioactivity eluted in the breakthrough fractions, the remaining 70% eluted in fractions containing structures with one (25%), two (37%) and three (8%) charges (Fig 3, inset). Treatment of the charged fractions with Vibrio cholerae neuramindase, in all cases resulted in a shift of the majority of the radioactivity to the column break-through fractions and remaining charged radioactivity co-migrating with the free sialic acid, upon paper chromatography, suggesting that sialic acid is the only charged substituent found on the N-glycans on HL-60 PSGL-1 (Data not shown). About two thirds (69%) of the radioactivity recovered from the P-6 separation of the peptide: N-glycosidase F released structures eluted as a broad cluster of peaks, starting at V0 and reaching through most of the column's fractionation range (Fig 3). When this radioactivity was pooled and analyzed on the GlycoSe-t quencer, a series of peaks with elution volumes ranging from V0 to ap-

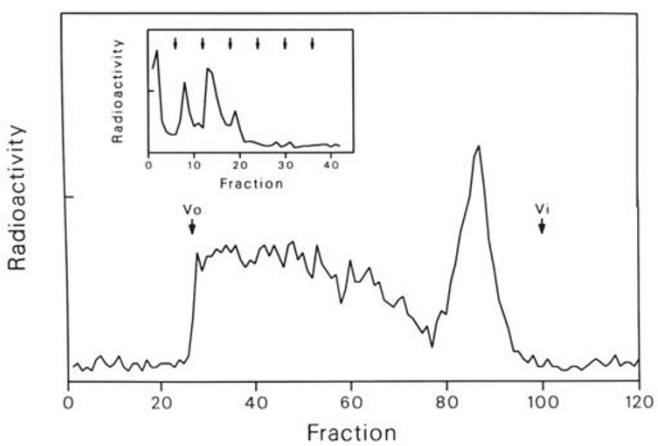

Fig 3. Size-exclusion and ion-exchange chromatography of N-linked oligosaccharides isolated from PSGL-1. [3H]-Glucosamine labeled N-linked oligosaccharides, isolated from PSGL-1 as outlined in the legend to Fig 2, were digested extensively with Arthrobacter ureafaciens neuraminidase and separated on a BioGel P-6 column (1.6 × 100 cm) equilibrated in 100 m M ammonium bicarbonate, pH 8.0. Aliquots from each fraction were counted for radioactivity. Inset: An aliquot of the total N-linked oligosaccharides recovered from PSGL-1 was separated on QAE-Sephadex as outlined in Materials and Methods. The arrows indicate (from left to right) the elution of bound radioactivity with 20, 70, 140, 200, 250 and 1000 mM NaCl.

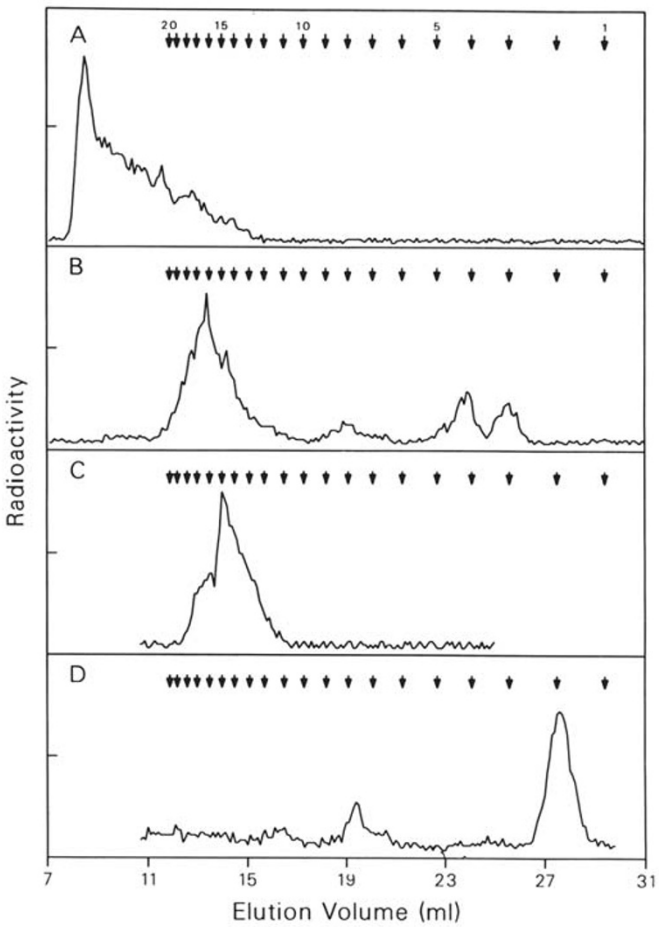

Fig 4. Sequential glycosidase treatment of the N-linked oligosaccharides on PSGL-1. Total N-linked PSGL-1 oligosaccharides were isolated as outlined in the legend to Fig 2. Sialic acids were removed by Arthrobacter ureafaciens neuraminidase treatment and the resulting neutral structures were separated on a Glycan Sizing Column. Panel A, separation of total, untreated N-linked oligosaccharides. Panel B, Separation of products generated by digestion of the total N-linked oligosaccharides with Bacteroides fragilis endo-β-galactosidase. Panel C, products generated by treatment of the approximately 16 GU fragment produced in the experiment shown in Panel B, with jack bean β-galactosidase. Panel D, products generated by treatment of the approximately 16 GU fragment produced in the experiment shown in Panel B with a mixture of jack bean β-galactosidase and jack bean β-N-acetylglucosaminidase. The numbered arrows indicate the elution volumes of dextran standard oligomers composed of 1-20 glucose units.

proximately 14 glucose units (GU), were observed (Fig 4, panel A). Panel A in Fig 4 also shows that a large portion of the radioactivity eluted at, or close to V0, indicating the presence of predominantly large structures, possibly with extended outer chains. To investigate this possibility, the pooled N-linked oligosaccharides (V_0-14 GU, Fig 4, panel A) were treated with Bacteroides fragilis endo-b-galactosidase and re-chromatographed on the GlycoSequencer. This resulted in the disappearance of the entire cluster of peaks between V_0 and 14 GU and the generation of four major peaks eluting at 16, 8, 4 and 3 GU. The ratio of recovered radioactivity in these peaks was approximately 8:0.3:1:1 (Fig 4, panel B). This result is consistent with the apparent size of the structures and strongly suggest the majority of the N-linked structures on PSGL-1 contain elongated outer chains composed of poly-N-acetyllactosamine repeat units. The peaks at 4 and 3 GU represent the predicted fragments from endo-β-galactosidase degradation of a linear poly-N-acetyllactosamine structure(s): Galb1, 4GlcNAc β1, 3Gal and GlcNac β1,3Gal. The identity of the 8 GU fragment is unclear. It may represent an outer chain fragment containing (branching) substituents, such as fucose, that inhibited internal degradation by the endo-β-galactosidase[26]. Supporting this notion is the observation that this fragment is retarded upon chromatography on AAL-Sepharose (Fig 6, panel B; compare below). The peak eluting at approximately 16 GU likely contains fragments composed of a $Man_3GlcNAc_2$ core (with or without a core-fucose) substituted with N-acetylglucosamines and galactose, i.e. again the predicted fragments from endo-β-galactosidase degradation of the outer chains of poly-N-acetyllactosmine containing complex type oligosaccharides. Treatment of the radioactivity in 16 GU peak with jack bean β-galactosidase resulted in a one GU shift in elution volume for approximately 76% of the radioactivity, suggesting that a portion of the structures in the peak contain a terminal galactose (Fig 4, panel C). On the other hand treatment with a mixture of jack bean β-galactosidase and jack bean β-N-acetylglucosaminidase resulted in degradation of essentially all the structure (s) in this peak and the generation of two fragments, eluting at 8 and 2 GU respectively (Fig 4, panel D). Eight glucose units is the predicted size of a fucosylated $Man_3GlcNAc_2$ core and consistent with this, this fragment could be further degraded by β-mannosidase digestion (Data not shown). The 2 GU fragment represents the released GlcNAc. These results are consistent with the 16 GU peak containing Man3GlcNAc2 core structures substituted with N-acetylglucosamine and (to some extent) galactose. Unexpectedly, the ratio of recovered radioactivity in the 8 and 2 GU fragments was approximately 1:4.7 (Fig 4, panel D). It is not clear why this (distribution of radioactivity between the core and outer amino sugars) is lower than predicted. Assuming equal distribution of radioactivity in the component amino sugars in the structures, the predicted ratio for a $Man3GlcNAc_2$ core containing four outer N-acetylglucosamines, the maximum number allowed by the apparent size of the structure(s), is 1:2; fewer outer N-acetylglucosamines would yield a higher ratio.

To investigate the apparent fucose content of the N-linked oligosaccharides on PSGL-1 (see above), an aliquot of the total, pooled N-linked structures (fractions V_0 through 14 GU; see above) was separated on Aleuria aurantia lectin-Sepharose. Seventy six per cent of the applied radioactivity interacted with the lectin (Tab 1), approximately a third of which was retarded and the remaining two thirds bound and eluted with 1 m M fucose (Fig 5, panel A). This suggests that the majority of the N-linked oligosaccharides on PSGL-1 are fucosylated. Core fucosylated N-linked structures bind with high affinity to AAL-Sepharose[23]. Separation of an additional aliquot of the pooled N-linked oligosaccharides on the lectin column following treatment with almond meal a-fucosidase again resulted in approximately 75% of the radioactivity interacting with the column and an elution profile almost identical to that obtained for the untreated material (Tab 1 and Fig 5, panel B), suggesting only low levels (or the absence) of fucose conjugated in 1, 3 and 1, 4 linkages. By contrast, treatment of the pooled structures with chicken liver a-fucosidase prior to chromatography on the lectin produced a considerable shift in the distribution of radioactivity in the fractions eluting from the column: Only 10% of the applied radioactivity bound to the lectin (and was subsequently eluted with 1 mM fucose); the remain-

Tab 1. Separation of intact N-linked PSGL-1 oligosaccharides and oligosaccharide exoglycosidase digestion fragments on Aleuria aurantia lectin-Sepharose.

Loaded fraction	% of loaded radioactivity interacting with the lectin
Total intact N-linked structures	76
Total N-linked structures treated with almond meal α-fucosidase	76
Total N-linked structures treated with chicken liver α-fucosidase	10
16 GU fragment from endo-β-galactosidase treatment	78
8 GU fragment from endo β-galactosidase treatment	N.D.
4 GU fragment from endo β-galactosidase treatment	0
3 GU fragment from endo-β-galactosidase treatment	0

N.D., Not determined. Due to overlap between radioactivity retarded on the column and the radioactivity in the break-through fractions, the proportion of retarded radioactivity could not be accurately determined (see Fig 6)

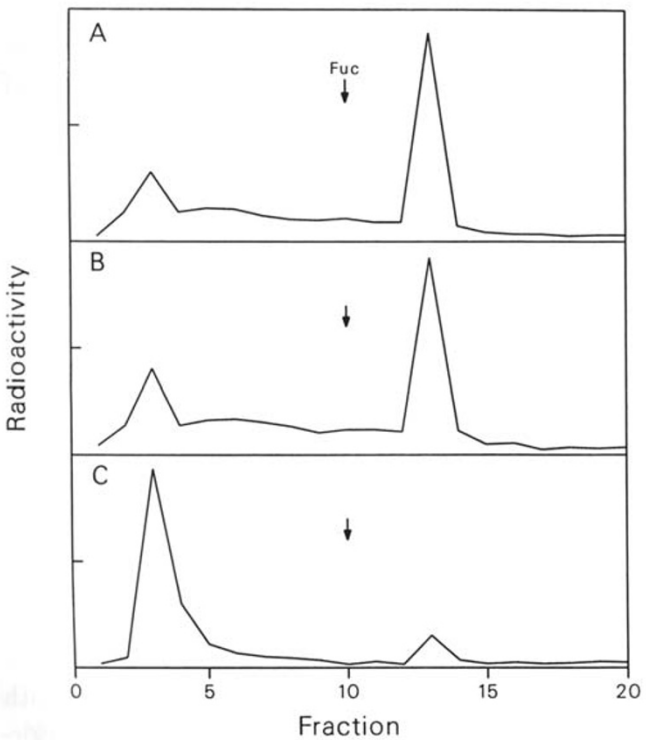

Fig 5. Aleuria aurantia lectin chromatography of N-linked oligosaccharides isolated from PSGL-1. Aliquots of the total N-linked oligosaccharides recovered from PSGL-1 (see legend to Fig 2) were separated on an Aleuria aurantia lectin-Sepharose column as outlined in Materials and Methods; bound radioactivity was eluted with 1 mM fucose (arrow). Panels A, B and C show chromatography profiles obtained from separations of the untreated radioactivity and radioactivity treated with almond meal and chicken liver a-fucosidase, respectively.

ing 90% was recovered in the column break-through fractions (Tab 1 and Fig 5, panel C). Further, chromatography of the approx. 16 GU endo-β-galactosidase degradation fragment(s) (see Fig 4) on the A. aurantia lectin resulted in an elution profile and distribution of radioactivity which was similar to that obtained when chromatographing the intact, undegraded structures (Tab 1 and compare Fig 5, panel A with Fig 6, panel A), while chromatography of the 4 and 3 GU fragments from this experiment resulted in essentially no radioactivity interacting with the lectin (Fig 6, panels C and D, respectively). Interestingly, chromatography of the 8 GU fragment resulted in a broadened peak close to the column break-through, indicating that this fragment may indeed contain fucose. Many structures containing outer chain fucose substitutions do not bind, but are retarded on AAL-Sepharose [23] and chromatography of a Le^x-containing standard oligosaccharide, Gal β1,4(Fuca1,3)Glc βNAc (1,3Galb1,4Glc-ol, resulted in a broadened elution peak virtually identical to that generated by chromatography of the 8 GU fragment, while chromatography of an unfucosylated LacNAc structure resulted in all the radioactivity being recovered in the bona fide break-through fractions only (Fig 6, panel B, inset, top and bottom panels, respectively). These results, although not conclusive, indicate that a portion of the N-linked oligosaccharides on PSGL-1 may contain fucosylated outer chains. The low levels of radioactivity recovered from the endo-β-galactosidase generated 8 GU fragment did not allow any further characterization.

Taken together the results presented above suggest that the predominant N-glycans on PSGL-1 are multiantennary, poly-N-acetyllactosamine-containing complex type structures. A large portion of the structures are sialylated and the recovery of structures containing three sialic acids suggests that some of them contain at least three outer chains, i.

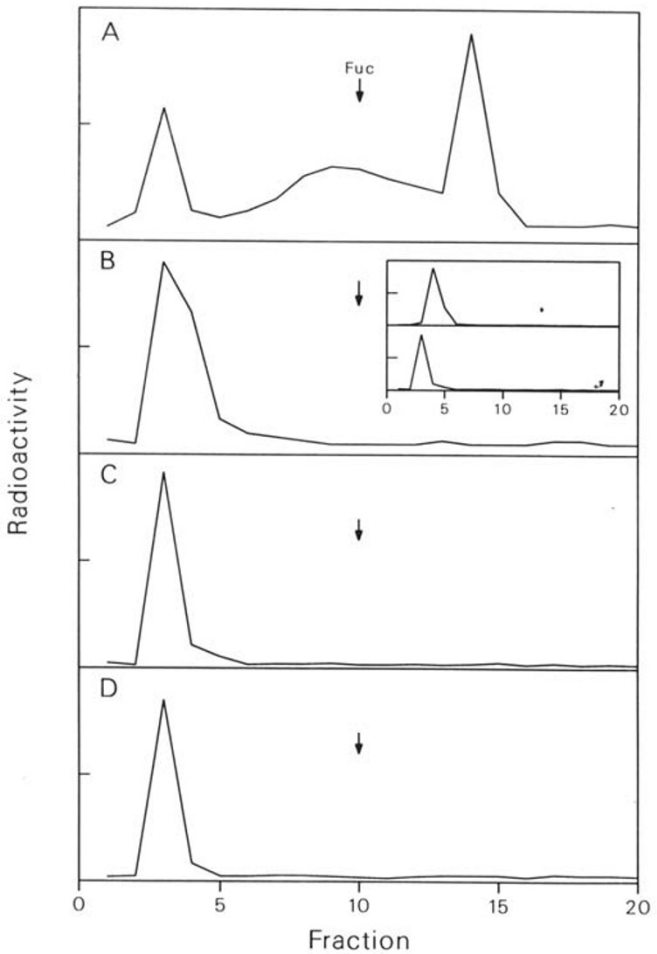

Fig 6. Aleuria aurantia lectin chromatography of fragments generated by endo-β-galactosidase treatment. Fragments generated by treatment of the total N-linked oligosaccharides isolated from PSGL-1 with Bacteroides fragilis endo-β-galactosidase (see legend to Fig 4) were separated on an Aleuria aurantia lectin-Sepharose column; bound radioactivity was eluted with 1 m M fucose (arrow). Panels A through D show separations of fragments eluting at 16, 8, 4 and 3 GU (on the Glycan Sizing Column), respectively. The inset in panel B shows separation of a standard Le^x-containing oligosaccharide and a standard LacNAc oligosaccharide (top and bottom panels, respectively).

e., triantennary structures. The majority of the structures are core-fucosylated and a smaller portion may also contain fucosylated outer chains. The susceptibility of the fucosylated structures to chicken liver a-fucosidase but not to almond meal a-fucosidase indicate that the core fucoses probably are conjugated in a 1,6 linkages to the reducing N-acetylglucosamine.

DISCUSSION

The data presented in this report suggest that HL-60 PSGL-1 contains predominantly sialylated multiantennary complex type N-linked oligosaccharides. The results from Concanavalin A chromatography together with the generally large size of the structures (>14 GU) is consistent with essentially all N-glycans on the molecule being larger than a biantennary complex type structure; in fact the distribution of a large portion of the radioactivity close to V_0 in the GlycoSequencer profile, suggests that the majority of the structures are considerably larger. This observation, in turn, is consistent with the poly-N-acetyllactosamine content of these structures, as determined by their susceptibility to digestion with endo-β-galactosidase. It is noteworthy that essentially all the radioactivity in the cluster of peaks generated by the initial separation of the structures (Fig 4, panel A), appears to be susceptible to endo-β-galactosidase treatment. This suggests a strong predominance of extended (poly-N-acetyllactosamine containing) structures on PSGL-1, consistent with the reported capacity of hematopoietic cells, including cells of myeloid lineage, for synthesizing poly-N-acetyllactosamine-containing oligosaccharide structures[27]. Although the majority of the N-glycans on PSGL-1 are fucosylated, the bulk of this substituent appears confined to the core portion of the structures. This observation, together with the apparent resistance of the fucoses to treatment with almond meal α-fucosidase, suggests that the majority of the structures do not contain $Lewis^x$ and/or $Lewis^a$ type substitutions. However, as discussed above, the 8 GU fragment generated by endo-β-galactosidase treatment, was retarded on AAL-Sepharose, indicating that a small portion of the N-glycans on PSGL-1 may indeed contain fucosylated outer chains. Whether these putative fucosylated structures are of functional importance for selectin binding remains to be determined. Previous reports have described the N-glycans on PSGL-1 as dispensable for binding[28] and a considerable body of evidence suggest that the selectins bind to O-linked oligosaccharide structures on PSGL-1[7-10], [14]. A specific threonine residue has been identified in the NH2-terminal portion of PSGL-1 that is believed to carry the (O-linked) ligand oligosac-

charide recognized by P-selectin[29-31]. Moreover, results from recent investigations clearly demonstrate that a significant portion of the O-linked structures on PSGL-1 are fucosylated[18], [19], thereby lending further support the involvement of O-linked oligosaccharides in selectin binding. Consequently, it is likely that at least P-selectin binds to fucosylated O-glycans on PSGL-1. On the other hand, P- and E-selectin reportedly bind to distinct sites on PSGL-1[9],[26]. Moreover, N-linked oligosaccharides have been implicated, repeatedly in E-selectin binding[4-6]. And it has been demonstrated that binding of E-selectin to PSGL-1 can be abolished by peptide: N-glycosidase F digestion[32]. Finally it is also noteworthy that Patel et al., when separating a total oligosaccharide structure preparations isolated from human neutrophils and U937 cells on an E-selectin column, found that exclusively N-linked oligosaccharides with extended, fucosylated outer chains bound to the column[6]. These independent but convergent findings all support the possibility that the fucosylated N-linked structures may be involved in the binding of E-selectin to PSGL-1. In conclusion, the N-glycans, in analogy with the O-linked structures on PSGL-1, contain the poly-N-acetyllactosamine feature previously reported on ligands for E-and P-selectin. A significant portion of the structures may also contain outer chains substituted with fucose. Additional experiments will be needed to determine whether the N-linked oligosaccharides on PSGL-1 have a function in the interaction with the selectins.

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